

SEX DETERMINATION IN THE WHITE-FLY
(TRIALEURODES VAPORARIORUM)

BY
FRANZ SCHRADER

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY, IN THE FACULTY OF PURE SCIENCE,
COLUMBIA UNIVERSITY

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FOUR PLATES (THIRTY-SIX FIGURES)

INTRODUCTION

Parthenogenesis in the Homopteran family of Aleyrodidae has been known for some years. In 1903, A. W. Morrill, in the course of some breeding experiments with *Trialeurodes vaporariorum*, found that all of the eggs laid by virgin females gave rise to males. Later, working with E. A. Back ('11), he discovered that the same held true for *Dialeurodes citri*, another member of the same family. Both of these cases were reported from America. In apparent contradiction to these conclusions, E. Hargreaves ('14), working on *Trialeurodes vaporariorum* in England, found that there only females emerge from eggs laid by virgin females.

Recently, C. B. Williams ('17) confirmed Hargreaves' observations in regard to the English form, but while on a trip to America also found the reports of the American investigators to be correct, basing both of these conclusions on sex ratios obtaining in random collections. In England, these collections were made in various greenhouses, and of some 500 specimens obtained, only $2\frac{4}{5}$ per cent were males. The American collection was made in Virginia, and there $36\frac{1}{2}$ per cent of 254 specimens were males. My own collections, made in the vicinity of New York City, showed that the males constituted $46\frac{4}{5}$ per cent of a total of 403. These counts are a sufficient indication of the fact that the earlier observations in regard to parthenogenesis in the species are not erroneous and that the parthenogenetic behavior is different in the two countries.¹

¹ N. R. Stoll and A. F. Shull, *Genet.*, vol. 4, in more extensive breeding experiments confirm the production of males from virgin females and find that the ratio of sexes in offspring from mated females is variable.

The only doubt which may exist arises from the question whether the same species is concerned in both countries. In this connection, A. C. Baker, of the U. S. Bureau of Entomology, having gone over some of Williams' English specimens, assures me that they are morphologically indistinguishable from the species occurring in America. We are therefore concerned with two lines of a species, which differ in their parthenogenetic behavior.

That these lines or races are not strictly confined within definite geographical limits may be indicated by an exceptional occurrence at Merton, England, where Williams found that over 34 per cent of 287 specimens were males. He believes, however, that they may have been introduced with some recently acquired plants. Unfortunately, all the families raised from virgin females of these exceptional white flies died before their sex could be determined, and no subsequent breeding experiments were made. I have not found the converse case to this in my work here, but think it perfectly possible that an occasional strain of parthenogenetic female producers may exist.

Very few observations have been reported on the offspring of mated females. Williams succeeded in raising two very small broods from mated females which came from the exceptional Merton strain. In these broods, as in broods which I have raised from mated American females, both sexes were represented. Mr. Williams informs me that he never observed copulation in specimens of the ordinary English stock. The broods produced by females used in such experiments were always exclusively female, but, since it is questionable whether fertilization had taken place, no conclusions can be reached from such data.

Summed up, we thus have the following:

1. *Trialeurodes vaporariorum* has two races, one of which gives rise by parthenogenesis to males (America), the other to females (England).
2. Mated females of the male producing race give rise to both sexes.

The present work has been confined to the race occurring in America. I am indebted to Prof. E. B. Wilson and Dr. A. H. Sturtevant for aid and encouragement.

TECHNIQUE

Adults and all the younger developmental stages can be obtained without difficulty from a great variety of plants, especially from members of the group of Solanaceae. In order to obtain eggs for the study of maturation, I found it best to put a number of females into a vial containing a small leaf of one of their food plants. If previously starved for half an hour, they will readily settle on the leaf to feed and lay eggs, which after a little practice can then be picked off under a binocular by means of a sharp curved needle.

The eggs were fixed best with Gilson-Carnoy's fluid (equal parts of chloroform, absolute alcohol, and acetic acid, saturated with sublimate), their chitinous shell making other agents practically valueless. Their diminutive size makes puncturing almost impossible. The spermatogenesis, best studied in pupae of the third instar, offers considerable difficulty, arising from the smallness of the cells and the clumping of chromosomes. The fluids of Flemming and Hermann—used both singly and in conjunction with urea—and the fluids of Carazzi, Champy, Bouin, and Gilson-Carnoy, sublimate acetic, alcohol acetic—with various modifications of these in respect to dilution and temperature, were tried with little success. Gilson's fluid gave a few good preparations, while the Bouin and urea method of Allen was more consistent and successful than any of the others. The study of fresh material with aceto-carmin offered no advantages. I could find no differences in fixation due to the various methods of killing. In any case, it is absolutely necessary to make an incision close to the gonads, and preferably on both dorsal and ventral sides. Unless this is done, penetration of the fixing agent is always poor.

Sections of 6μ were found best for the study of the maturation phases in the eggs, while sections for the spermatogenesis were cut 3μ to 5μ thick. Hematoxylin without a counterstain proved to be the best stain.

MATURATION IN THE EGG

The earliest stages of maturation are found in the adult female, the abdomen of which is best fixed and sectioned entire. During the growth stages, the nucleus is situated at the center of the egg. Its black nucleolus, surrounded by an unstained protoplasmic area, and its definite nuclear wall make it readily distinguishable from the mass of densely staining yolk granules which fill the egg at this time. The prophase offers nothing that is new, the chromatin going through the various phases of chromosome formation while the nucleus approaches the central periphery of the egg. Here the nuclear wall disappears, and further developments take place in a clear irregular area of protoplasm, as in many other insect eggs.

Tetrads were found in a few of the preparations, their appearance resembling those of typical Homoptera as described by Miss Boring ('07). They are eleven in number, and their further condensation gives rise to the rounded deeply staining bodies which occur in equatorial plates of the first division. Although differing only very slightly in size, their arrangement in the plate seems to be a very definite one, and is the same in all eggs. Normally the metaphase is the farthest stage reached while the egg is in the body of the insect, but occasionally early anaphases are found in females which have been prevented from laying for a short period.

In the first division, each of the chromosomal bodies undergoes division and eleven dyads go to each pole. The outer group of these, or first polar nucleus, is not extruded, but remains close to the periphery. Both the polar nucleus and the egg nucleus enter immediately on a second division. The division of the former appears to lag somewhat behind, as the irregular arrangement of the dumb-bell-shaped dyads in figure 7 and in other preparations would indicate, but two daughter bodies are formed from it in any case. The division of the nucleus proceeds normally. The resulting second polar nucleus, like the first now divided, is not extruded, but remains close to the periphery, where all three ultimately disintegrate, while the reduced egg nucleus, with its eleven univalent chromosomes, travels toward the center of the egg.

PARTHENOGENESIS

Up to this point all eggs behave in exactly the same manner. The subsequent processes in the egg-nucleus depend on whether the egg has been fertilized or not. If no spermatozoon has entered the egg, a well-defined nuclear wall is formed around the matured egg-nucleus, and the chromosomes enter a semi-resting stage in which they become less sharp and definite in outline. They, nevertheless, still can be counted. Soon after, the rounded form is lost and the chromosomes lengthen, assuming the shape typical of those found in the dividing somatic cells of all the succeeding developmental stages. The first segmentation division proceeds normally, each of the first two nuclei receiving eleven chromosomes.

FERTILIZATION

If the egg has been fertilized, the egg-nucleus, as in the unfertilized egg, likewise approaches the center. The condition and position of the spermatozoon previous to this stage is very difficult to determine. Various sections showed what may possibly be the sperm head in a resting condition, but identification is never certain, because of possible confusion with the equally stained yolk material. Now, however, the sperm nucleus becomes apparent. At first still retaining the lengthened and pointed form of the sperm head, it gradually rounds out, undergoing the ordinary process of chromosome formation. When the two pronuclei meet, they are equal in size and each shows eleven lengthened chromosomes. The nuclear walls then break down and the twenty-two chromosomes are inclosed in an irregular vesicle. The first segmentation is regular in character, as before, and results in two nuclei, each with twenty-two chromosomes.

THE CHROMOSOMES SUBSEQUENT TO MATURATION AND
FERTILIZATION

Cytological investigation of such forms as *Ascaris* and the bee has demonstrated the possibility of formation of multiple chromosomes, that is, products of the union of two or more chromo-

somes, which are found during maturation and fertilization. These then appear as single chromosomes, but betray their compound character later when they break up into the component units again. In view of this fact, I traced out the behavior of the chromosomes in the present case with some care.

In the fertilized egg with its twenty-two chromosomes, the first segmentation division is followed by others which are perfectly regular. The first nuclei, still situated in the interior of the egg, all show twenty-two chromosomes. After the formation of about twelve such nuclei, these approach the periphery, and further divisions there establish the blastula. In these early stages, and up to the late blastula, division of all the cells occurs at practically the same time. In favorable preparations, therefore, the chromosomes in a great many cells of an embryo can be counted. One of my preparations shows such a condition, and the number can be made out definitely as twenty-two in some cases, and at least closely estimated to be such in many other cells. This constancy in the number of chromosomes in any single embryo is found also in the gastrula stage, where, however, divisions do not occur so rhythmically and the cells are smaller. Finally, pupae representing the various instars frequently show a number of cells in which the chromosomes are countable, and if twenty-two chromosomes are seen in one cell, every other cell in which the chromosome number can be made out will show the same number. Older pupae, that is, those in the fourth and latter part of the third instar, having the gonad in an advanced stage of development, always prove to be females in such cases, as an examination of the reproductive organs will show. I have never been able to make an absolutely certain count of the oogonial chromosomes, although very good estimates can be made in some cases. The number is here also twenty-two to all probability—certainly more than eleven.

The unfertilized egg, like that which has been fertilized, shows no irregularities following the maturation divisions and the smaller number of chromosomes makes their counting easier. As before, after the first few divisions, which here show eleven chromosomes, the nuclei travel to the periphery and the blastula

is formed. The latter, as well as the gastrula and the various pupal stages, show perfect constancy in the chromosome number. And in this case, finally, the older pupae, which show eleven chromosomes in the somatic cells, are found to be males with testes partly or fully developed. The only exception to the constancy in the number of chromosomes of the various somatic cells is furnished by the pseudovitelline cells or mycetoma, to which I will refer later.

Although I realize fully the danger of laying too much stress on chromosome counts of somatic cells, their remarkable constancy and definite coincidence with sex in this case cannot be altogether without significance.

SPERMATOGENESIS

The spermatogenesis presents considerable difficulty, due chiefly to the diminutive size of the cells and the tendency of the chromosomes to clump. In the hope of finding more favorable material, I also investigated a number of other genera and species of Aleyrodidae, comprising the following: *Trialeurodes coryli*, *T. packardi*, *T. morrilli*, *Tetraleurodes mori*, *Aleurochiton forbesii*, and also unidentified species from the common ragweed (*Ambrosia artemisiifolia*), wild lettuce (*Lactuca canadensis*), *Viburnum acerifolium*, and *Eupatorium purpureum*. None of these, however, offered better conditions than are to be met with in *Trialeurodes vaporariorum*, and, on the whole, very little difference was observed.

I found it impossible to count the chromosomes in those phases which are undoubtedly spermatogonial. However, the mitoses are evidently normal. The equatorial plates of the various spermatogonial divisions observed are regular and identical in size.

The main problem of course centers in the question of how the haploid number of chromosomes is retained in the male during maturation. As is well known, Hymenoptera, in which parthenogenesis is haploid, simply eliminate the reduction. An attempt is indeed made to initiate the process, but the chromosomes do

not separate, and only a protoplasmic bud is constricted off as a result. The next and last division, then, has the nature of an equation division. We may probably conclude also in *T. vaporariorum* that the last division is equational. By analogy with the Hymenoptera, it should be the division preceding this last which is the critical one, but, apparently, there is nothing of exceptional character in the division concerned, for, as far as can be determined, division plates here are exactly like those found in the spermatogonia.

As in all other divisions, the most advantageous stage for counting the chromosomes is found just prior to the arrangement of the chromosomes into the equatorial plate. They are then irregularly distributed, but also less clumped, whereas they form a dense mass as soon as they have entered the plate. Eleven chromosomes can be made out. They are lengthened and identical in appearance with those observed in the somatic cells. Preparatory phases of this division show no trace of tetrad formation. Finally, the telophases show what are, in all probability, equal masses of chromatin in each daughter cell, and there is certainly no abortive division at this time. As indicated above, it is to all intents and purposes a spermatogonial division.

In spite of careful search, I could find no growth state preceding the final division, although it seems improbable that such a phase should be lacking. The daughter cells resulting from this division receive apparently equal masses of chromatin. The number of chromosomes prior to division cannot be made out with certainty, but must be very close to eleven, while the number after the division cannot be estimated. This, then, is probably the ordinary equation division of maturation.

It would appear, therefore, from the foregoing that there is in *T. vaporariorum* no abortive division, and that the first spermatocyte division has been entirely suppressed. This conclusion is indicated also in another way. The spermatogonial as well as the spermatocyte cells of the various stages are always assembled in definite groups, and in most cases show a radiating arrangement around a common center which seems to indicate that they originate from a single cell. All the cells of any one group are at

practically the same stage of development, so that they can be easily distinguished from the cells of neighboring groups, which are generally at different stages. In addition, all except the earlier spermatogonia, clearly show cyst walls. Since the cell number is practically identical in all cysts which are at the same stage of development, it is possible to count the cells after each division, and thus determine whether a certain percentage is lost. If the number of spermatozoa is proportional to the number of spermatogonial cells, then it can justly be concluded that no abortive division has occurred and that all divisions have resulted in functional cells. That such is indeed the case seems to be pointed out by the following:

Spermatogonial cysts show 7 to 8, 13 to 16, and 28 to 33 cells.

Cysts with cells after the first of the last two divisions show 52 to 59 cells.

Cysts with cells after the final division or spermatids show 103 to 111.

Cysts with ripe spermatozoa (which are easily counted when the sperm bundle is cut at right angles) show 101 to 123.

These figures show, as is to be expected, that a few cells are lost during development. But, certainly, there is no such essential loss as in case of the ant *Camponotus* (Lams, '08), where each spermatocyte produces but two functional spermatozoa, or the bee, where only one is finally produced. The objection may be made that not all of the spermatozoa of *T. vaporariorum* may be functional, and a large percentage may be rudimentary—assuming that the numerical proof given is the only evidence available. The only answer that can be made here is that spermatids and spermatozoa, respectively, show no differences of size or structure.

Comparing the phenomena here described with those seen in the nearly related groups of Aphids and Phylloxera, it will be seen that in those insects the chromosomes of the somatic cells have a very characteristic oblong or else lengthened shape. With a few exceptions, this form is lost in the maturation divisions, where the chromosomes become rounded and lumplike. This is true of both sexes. Exactly the same feature is noticed in the female of *T. vaporariorum*, the chromosomes of which seem to

follow the same transformations witnessed in the other groups mentioned. In contrast to this, the male, which in its somatic cells has chromosomes shaped like those found in the female somatic cells, shows no corresponding change in the maturation phases. The lengthened form is retained through all the divisions. This may have no significance, though it does seem to indicate an exceptional condition.

Judging from the evidence at hand, it seems probable that the reduction division has been eliminated altogether. It may be suggested that the division preceding the final one is also equational, and that since there is basically no difference between a spermatogonial and an equational division, we are concerned merely with a question of names. But our present knowledge of the meaning and mechanism of the ordinary equational maturation divisions is not sufficient to make such a statement altogether safe. Furthermore, the assumption of two equational divisions in maturation meets with serious difficulties arising from our present conceptions of the significance of maturation—a point which will be discussed at the end of this paper.

THE OCCURRENCE OF *TRIALEURODES VAPORARIORUM* IN ENGLAND

As I have noted in the introduction, although Williams apparently assumes that normal females of the English line produce both sexes when fertilized, such a fact remains to be experimentally established. Those of his breeding experiments which proved successful were made with the exceptional Merton stock which resembles the American line in its sex-ratios, and is therefore not of the true or normal English type.

The parthenogenetic production of females can be explained by an omitted reduction division, a reunion of one of the polar bodies with the egg-nucleus, or a doubling of chromosomes at some stage of development. So far as we know, the sperm nucleus of animals does not attempt union with the nucleus of the egg until the latter has undergone the maturation process. I think therefore, that it is only fair to assume that the spermatozoon may very well enter the egg of mated females of the English race, but that it plays no further rôle unless reduction takes place.

The fact that males do sometimes occur in English collections may mean that a few representatives of the so-called American race exist here and there or else that there is at times an irregularity or reversion to normality in the maturation divisions.

Should it be shown definitely that the English line does indeed produce a mixture of sexes from the mated females, we would have to take recourse to an explanation based on some such chromosomal behavior as is known in the Hemiptera and Orthoptera. To explain it on the basis of the cytological evidence from the American line or on the basis of the chromosomal behavior as found in the Hymenoptera, we would have to resort to the somewhat far-fetched hypothesis that there are two kinds of spermatozoa, both of which, on entering eggs, cause them to undergo reduction, one of these spermatozoa being otherwise non-functional, so that the egg fertilized by it is left with the haploid number and produces a male. If the other kind of spermatozoon then be assumed to be normal and to restore the diploid condition in the egg it has entered, such an egg would produce a female. An analogous case, where the spermatozoon enters the egg, starts development, but plays no further part, and finally disintegrates, is found in *Rhabditis aberrans* (Krüger, '13).

The origin of the English race—the American line representing the normal or parent stock (Williams, '17)—may be due to environmental influence. It is conceivable that new physical influences of a hitherto unoccupied region may in some way act on the egg so as to modify or suppress the polar-body formation. But the question immediately arises why these same influences should not also act on the eggs of such a strain as was found at Merton and which was evidently not of the English type.

A more acceptable hypothesis would be based on an origin by mutation. Reciprocal crosses between the English and American lines should make it possible to determine whether the problem rests on a Mendelian basis. Once a female-producing line had become established, it might, and probably would, displace a male-producing line, as has been pointed out by Williams ('17) in some mathematical considerations of the problem. This would be especially true in a new country where the distribution

of the animal is limited to a small area at first. Crowding out of one line by the other would progress very fast in that case.

SEX RATIOS

Knowing that unfertilized eggs give rise to males in the American race, it is to be expected that, in a random collection, there would be a preponderance of that sex. Such is not the case, however, and this is due, no doubt, as Williams has already pointed out, to a difference in the duration of life of the two sexes. In one of my breeding experiments the female lived twenty-five days, while the males were generally dead by the tenth or eleventh day. Hargreaves ('14) mentions an adult that lived thirty-eight days, but does not give the sex of this specimen. He notes further that the duration of life is to some extent dependent on temperature, cold weather "making them live more slowly."

Judging from the cytological evidence, the production of the two sexes seems not to be due to two kinds of spermatozoa, but to be dependent merely on fertilization or its omission. Like the earlier observers, I found that virgin females produced only males, eight broods ranging from eight to fifty-two individuals having been raised to confirm this evidence. Mated females produced both sexes, and the proportion of these was very variable. This irregularity in sex ratios is open to two possible explanations: either the female has control over the spermatheca and, reacting to some stimulus, permits each egg to be fertilized or not, as the case may be, or the spermatheca acts perfectly mechanically and every egg is normally fertilized until the supply of spermatozoa is exhausted. In either case, the sex-ratio would be liable to great variation.

To determine this, I mated virgin females and kept a careful account of their offspring. I found that the eggs first laid were also the first to hatch and develop to the adult stage. In doing this, I did not keep track of every egg laid—a physical impossibility—but only of the batches deposited on successive days. If the female has control over the spermatheca, it is probable that the offspring will belong to either sex from day to day, the first eggs giving rise to a mixture of sexes. If, on the other

hand, every egg is fertilized until the spermatozoa are exhausted, the first eggs should all produce females, and after a variable period, all eggs should result in males.

I experienced the same difficulties as Williams in his breeding experiments. The small size and peculiar delicacy of the adults causes the handling of isolated individuals to result in many accidents. As the results in the following table seem to indicate, the female can control fertilization, and probably some stimulus determines whether spermatozoa are liberated or not whenever an egg is laid.

Order of emergence on successive days

	I	II	III	IV	V	VI	VII	VIII
a	2 ♀, 1 ♂	7 ♀, 4 ♂	5 ♀, 16 ♂	3 ♂	1 ♂	2 ♂	3 ♂	1 ♂
b	1 ♀	17 ♀, 1 ♂	3 ♀	5 ♂	7 ♂			
c ¹	2 ♀	3 ♀						
d	3 ♀, 1 ♂	1 ♀, 2 ♂	5 ♀	5 ♀, 2 ♂	3 ♀, 1 ♂	4 ♀, 8 ♂	4 ♂	
e	2 ♀	1 ♂						
f	1 ♀, 4 ♂	2 ♀, 4 ♂	3 ♀, 1 ♂	3 ♀, 1 ♂	2 ♂	1 ♀		

¹ The mother died on the third day after starting to lay.

THE MYCETOMA CELLS

The pseudovitelline or mycetoma cells are easily seen in the living pupae as two yellow masses situated just posterior to the midline. From five to eight of these cells are taken into each egg before laying, but their function is problematical, for they never seem to be used in the nutrition of the embryo. Occasionally a duct evidently connecting the cell mass with the exterior can be made out in the sections, so that possibly they may have a secretory function. Buchner ('18) has called attention to these cells as hosts of symbiotic 'fungi,' and has traced out their history in some detail.

As I have mentioned previously, in these cells the number of chromosomes is found to be irregular. Generally there are from thirty to thirty-five chromosomes, the number being independent of the sex of the individual. Such irregularities have often been observed in similar cells of other animals, and mitosis without

cell division and other abnormalities are not uncommon in such structures.

A very striking feature observed at certain stages is the presence of very definite tubules interspersed through the cytoplasm. These may stand in some relation to the symbionts described by Buchner, although the latter apparently did not see them in the European species he examined. They are in some respects very similar to Holmgren's canals, but, unlike the latter never extend to the periphery of the cell. In division they are placed immediately around the spindle. Their distribution seems haphazard at this stage. After division they appear to break up into granules, only to be reformed immediately afterward, but I am not prepared to maintain that this phenomenon may not be due to fixation.

I made some attempts to determine the nature of these structures—whether mitochondrial or otherwise. The poor preservative action of osmic mixtures when used in connection with these cells eliminated the Benda stain at the outset. The mixture of formalin and potassium bichromate employed by Kopsch gave very good fixation at times, and, when used in conjunction with Kull's modification of the Altmann stain, gave some excellent preparations. Here, as in certain gland cells of *Myxine*, recently studied by Schreiner ('16), the nucleolus and the thread-like inclusions stain red, while the chromatic material is a faint green. Such a stain is not very specific, however, for, as Professor Wilson pointed out to me, the acid fuchsin is apt to stain any cytoplasmic inclusion, regardless of its nature. Janus green used on fresh material did not stain the structures in question, but such negative evidence does not carry much weight. The tubules stained a deep black with haematoxylin. When fixed with the Gilson-Carnoy fluid, part of the tubule or what may be called the matrix disappeared, leaving a spiral skeleton or framework which retained the haematoxylin stain and still showed the tubular structure. Such pictures give the impression that they are composed of two different substances, only one of which is dissolved by the action of the strong fixing agent.

Schreiner's description of the origin of cytoplasmic granules in case of *Myxine* finds one curious parallel in these cells. Very often a cell will show three or more nucleoli, which, judging from their size, have originated from the normal single nucleolus. In many cells the nucleolus is seen dividing, and in others a sort of budding is observable. In the latter case, smaller masses of nucleolar material are visible in the nucleus, some just forming from the parent body, others separated from it by variable distances, but still connected with it through a faint but definite thread, and still others near the periphery of the nucleus and entirely independent of the nucleolus. I was unable to find such bodies still connected with the nucleolus, but outside of the nuclear membrane. In every instance where such an interpretation might have been made, the alternative of incorrect focusing or confusion with cytoplasmic inclusions of a different nature could be held. To eliminate the latter possibility, I tried the expedient of destaining very strongly. Since the nucleolar material retains the hematoxylin after the other cell constituents have become decolorized, its presence outside of the nucleus could then be determined with more certainty. But although the nucleolar material appeared perfectly sharp and distinct inside of the nucleus in such cases, none of it could ever be found definitely outside.

DISCUSSION

Without attempting to give here any general review of the great literature relating to parthenogenesis, I will briefly indicate certain difficult and still obscure points which seem to call for further investigation. It must be remembered throughout that parthenogenetic eggs belong to either of two groups—one in which they are incapable of fertilization and development is asexual in the true sense; the other in which the eggs may be fertilized or not, development proceeding in either case. Usually the unfertilized eggs of this last group then develop with the haploid number of chromosomes.

The researches of Schleip ('08), Nachtsheim ('13), and others have now well established the fact that in the majority of Hymen-

optera the sexual eggs normally undergo two maturation divisions, in the course of which the chromosome number is halved. As in case of *Trialeurodes vaporariorum*, such eggs will develop into males if unfertilized, but if the diploid number is restored through the entrance of a spermatozoon, a female is developed.

Although haploid parthenogenesis of the sexual eggs thus appears to be normal among Hymenoptera, there appear to be cases where certain eggs are asexual, parthenogenetic development taking place with the complete diploid number of chromosomes. As might be expected, such eggs give rise to females. This is found most generally in species where there is an alternation of sexual and parthenogenetic generations. I need mention only the gall-fly, *Neuroterus lenticularis* (Doncaster, '10, '11, '16), where the spring generation consists solely of parthenogenetic females, while the summer generation is represented by males and sexual females. The eggs of the latter undergo maturation and reduction like those of ordinary Hymenoptera, and since these eggs must be fertilized in order to develop, they always give rise to females (the parthenogenetic females mentioned above). The parthenogenetic females, however, produce two types of eggs. Those which give rise to the males again conform to the normal in that reduction takes place and parthenogenesis is haploid, while in those eggs from which females are developed, the diploid number is retained. Doncaster believes that there no polar bodies are given off. These proceedings are thus apparently clear, but what seems to be a more exceptional case is furnished by *Rhodites rosae*. No males have been found in this species by cytological investigators, and parthenogenesis is without doubt obligatory. Judging from analogous cases, it might be expected that such parthenogenesis is diploid, but analysis of the case presents various difficulties. Henking ('92) and Schleip ('09), both of whom have investigated maturation in these eggs, agree that two polar bodies are given off. Henking regards meiosis as normal, the chromosomes being reduced to the haploid number. The diploid number is, however, then restored in the first cleavage through a doubling of each chromosome.

If this account be correct, the case presents no difficulties, but Schleip states that he could find no such doubling of chromosomes after maturation. He believes that the chromosome number observed in the maturation is diploid and that this is kept constant because both maturation divisions are equational.

Such an explanation meets with serious difficulties, arising both *à priori* and from detailed observations on the meiotic phenomena, especially in case of the Hemiptera and Orthoptera. It has been established to a practical certainty that the reduction and equation divisions are perfectly definite in their operation. A fine substantiation of this rule is furnished, for instance, by *Trimerotropis*, an Orthopteron in which Miss Carothers ('17) has described several heteromorphic chromosome pairs, in which the synaptic mates differ distinctly in form. No correlation exists between the different pairs in regard to their distribution during reduction, so that a daughter cell may receive either member of such pair as a matter of chance. After separation, each homologue undergoes an equation division, and there is never a violation of this proceeding. A more special case is found in the 'm' supernumerary of *Metapodius* (Wilson, '09) and also the extra chromosome of *Oenothera lutea* (Gates, '14). Such supernumerary chromosomes may divide at either the heterotypic or homotypic division, but they never undergo more than one division. Finally might be mentioned the sex chromosomes, which are subject to the same rule. Before basing an explanation on the grounds of two equation divisions, therefore, a more sufficient proof than is given for *Rhodites rosae* and a few similar cases must be presented.

It is very possible that the problem involves the formation of multiple chromosomes. It was this most difficult point in Hymenopteran cytology which lay at the bottom of the long controversy concerning the bee. Nachtsheim ('13), who seems to have said the final word in this matter, offers the following explanation: The diploid number in the bee is 32, but through a coupling of these only 16 appear to be present in the oogonia. The reduced nucleus presents 8, and these must therefore be considered as bivalent. The case is complicated still further by the fact that 64 chromosomes appear in some somatic cells.

It is conceivable, therefore, that in *Rhodites*, also, there is at some stage a multiplication of chromosomes which restores the diploid number in the reduced nucleus. Perhaps this may occur in the oogonia, where Schleip seems to have made no exact counts. In one of the maturation divisions, the products of such a multiplication which have joined in pairs are separated from each other. This would then be a reduction division in the sense that paired whole chromosomes are separated from each other. The other division is then purely equational. Schleip's own observations show that phenomena such as have been described for the bee very probably do occur in *Rhodites* also, for he describes a pairing of chromosomes in the blastoderm nuclei, through which their number is temporarily halved. Furthermore, his figures of the first polar division show formations that may very well be tetrads, and do not speak at all against a reduction division.

Two equation divisions were also reported in the parthenogenetic eggs of the saw-fly, *Nematus ribesii*, by Doncaster ('07), but this interpretation has seemingly been abandoned, for Doncaster makes no mention of it in his "Determination of Sex" ('14).

Occasionally there are reports of cases which seem to contradict the general rule of chromosomal behavior among the Hymenoptera. Thus G. W. Onions* (Jack, '16) found that virgin workers of the Cape honey-bee gave rise not only to drones, but also to workers and queens. Such cases are only evidence for the fact that irregularities are apt to occur in the polar body formation, and that some races and species are more prone to such irregularities than others. Suppression of reduction or reunion of polar bodies with the nuclei offers the most plausible explanation in such cases.

The manner in which the males of the Hymenoptera retain their haploid complex of chromosomes during maturation is too well known to merit a long discussion. They all agree in having an abortive reduction division, which does not result in changing the chromosomal constitution. As regards the equation division, there seem to be two natural groups. In one, including *Apis* (Mark and Copeland, '06; Meves, '07), *Xylocopa*

(Granata, '09), and *Osmia* (Armbruster, '13), the equation division results in two unequal second spermatocytes, only one of which forms a functional spermatozoon. In the other, comprising *Vespa* (Meves and Duesberg, '07), *Neuroterus* (Doncaster, '07), and *Camponotus* (Lams, '08), two equal cells result, both of which form functional spermatozoa. The rudimentary spermatozoon produced in the first group is evidently due to unequal division of extranuclear material, for in both groups the division of chromatin appears to be equal and is no doubt truly equational.

The rotifers almost certainly belong to the groups in which parthenogenesis is haploid. Parthenogenetic eggs which give rise to other parthenogenetic females give off only one polar body and no reduction takes place. The sexual eggs give off two polar bodies and the chromosomes are reduced to the haploid number. If fertilized, the diploid number is restored and a female is produced; if not, the egg develops into a male with the haploid number (Whitney, '09). Exceptional conditions are met with in the spermatogenesis. Whitney ('17, '18) describes two kinds of spermatozoa in each male, one being large and motile, the other smaller and apparently non-motile, and these are present in the ratio of 2 to 1. He believes that both classes of spermatozoa contain chromatin, and concludes that the first maturation division gives rise to two cells, one of which forms the smaller spermatozoa without further division, while the other divides once more to produce two large motile spermatozoa. The latter class thus undergoes two divisions and, although Whitney does not commit himself definitely on this point, the conclusion once more presents the serious difficulty of two equation divisions, since males are haploid to start with. When based merely on Whitney's numerical data, such an explanation is very tempting, but no cytological evidence has yet been produced in its favor. The need for future study of the spermatogenesis is obvious. Taking Whitney's evidence as given, it is barely possible that the numerical ratio may result from the existence of two classes of spermatogonia, equal in numbers, but not necessarily externally distinguishable, one of which would give rise

to oligopyrene spermatozoa directly, while the other underwent an equational division and produced eupyrene spermatozoa. But such an explanation would rest on a very insecure basis, for the whole problem of abnormal sperm formation is still very much unsettled.

The breeding experiments of Hindle ('17) with lice, *Pediculus humanus*, may be also mentioned here. Hindle obtained from single females broods consisting in some cases of both sexes, in others of males or females exclusively. Males were always put together with the females, but copulation was not observed, Hindle evidently assuming that this always took place. It seems to me that this is a fairly clear case of haploid parthenogenesis.² The fact that the same female in one instance produced consecutive broods of all males and all females seems to point to fertilization as the determining factor. Very probably the amount of sperm in the spermatheca determines whether females exclusively or a mixture of sexes is produced, while the entire absence of spermatozoa results in the production of males. *Anthothrips verbasci*, in which A. F. Shull ('17) has found both sexes produced by fertilized females, and males only by virgins, no doubt also comes under the heading of haploid parthenogenesis.

There are some cases where sexual eggs, capable of parthenogenesis, apparently develop with the diploid number of chromosomes if unfertilized. The tick, *Ambylomma dissimile*, which is parasitic on Amphibia and reptiles, gives rise to females through parthenogenesis, while fertilized females produce both sexes (Bodkin, '18). The numbers given are too small to admit of any definite conclusion, but very probably parthenogenesis is diploid, and in fertilized eggs the spermatozoon influences polar body formation in some way.

Very similar cases to this are found among the Orthoptera, a group in which the normal chromosomal behavior in the causation of sex is well known, females being homozygous and males

² Since the time of writing, K. Foote, *Bio. Bull.*, vol. 37, and L. Doncaster and H. G. Gannon, *Q. J. M. S.*, vol. 64, have investigated the cytology of *Pediculus*. Indications of a rudimentary division in spermatogenesis seem to confirm the above, but the chromosome counts are inconclusive.

heterozygous for sex. While reproduction is sexual in the great majority of the order, there are some well-established cases of parthenogenesis on record. Thus *Saga serrata* is represented by both males and females in Asia Minor, but in southern Spain only females are ever found (Bolivar, '97). Hebard ('18) has not seen a single male among some thousand immature and adult specimens of the blattid, *Pycnoscelus surinamensis*, although a male has recently been reported by another entomologist. MacBride and Jackson ('15) raised three thousand offspring of the phasmid, *Carausius morosus*, all of which were parthenogenetically produced, and among this number observed only six males and one gynandromorph. These and many similar cases demonstrate that parthenogenesis occurs, that in some species it is practically obligatory, and that with very rare exceptions it gives rise to females. A few cases are, however, known in which asexual reproduction is facultative, and eggs will develop whether fertilized or not. As in the obligatory type, parthenogenesis here produces females, while fertilized eggs give a mixture of males and females. I need mention only the grouse locust, *Apotettix*, reported by Nabours ('19), and the phasmid, *Clitumnus* sp?, which was studied by Fryer ('13). Experimental genetic work was done in both cases, and this showed that segregation occurred in the first generation of parthenogenetically produced offspring. This is good evidence for the conclusion that reduction occurs in these unfertilized eggs as well as in the fertilized ones. A further confirmation of this was obtained when Nabours found that the F_2 parthenogenetic generation was homozygous for certain characters under observation.

Cytologically, the maturation phenomena in parthenogenetic eggs have been studied only in *Bacillus rossii*, where von Baehr ('07) found two maturation divisions. He seems to believe that both of these are equational divisions (Buchner, ('16), has also gathered this impression from von Baehr's account), and that therefore no reduction takes place. However, his figures of equational plates of the first division show tetrad-like structures, and since he is unable to count or even estimate the oogonial or somatic chromosomes, it is probable that here also reduction occurs as in sexual species.

To sum up, the eggs of all Orthoptera are subjected to reduction. Those which are parthenogenetically developed give rise to females, while those which are fertilized result in a mixture of sexes. How, then, is the chromosome number kept constant, and why, since a reduced egg has only one sex chromosome, is not a male developed from such eggs? As indicated in case of the tick, this question might find a satisfactory answer in the assumption that the spermatozoon affects the formation or behavior of the polar bodies, such that its entrance into the egg causes the second polar body to be cast out or disintegrate, sex being then dependent on the kind of spermatozoon which has entered. We might then assume that in eggs that are not fertilized, the second polar body fails to be extruded or, if formed, it rejoins the nucleus. In the former case, there would be a haploid complex of dyads, each of which breaks up into the component units at a later stage, while in the last-named case, the diploid number is immediately restored by the rejoining second polar body.

Parthenogenesis is also known in the Lepidoptera, and in this connection the Bombycidae and *Liparis dispar* might be specially mentioned. Platner ('88) thinks that two polar bodies are given off by parthenogenetic eggs of the latter, but unfortunately he was unable to rear offspring from these to the stage where sex can be determined, and previous experiments with the same species admit of no definite conclusion. Although cytological work on parthenogenesis has been followed out to only a very limited extent, some very interesting observations have been made on the maturation phenomena in hybrids, which seem to offer the most convincing evidence thus far produced of the occurrence of two equational divisions in maturation. Federley ('13), in his well-known crosses of *Pygaera* species, found that no synapsis takes place prior to maturation and, from both cytological observations as well as the fact that after two maturation divisions the number of chromosomes is still practically equal to the sum of the haploid numbers of the parents, concludes that two equational divisions must occur. The evidence given by Fed-

erley is so clear cut and definite that it seems as if this interpretation is the only one that can be made at the present time. Harrison and Doncaster ('14), who crossed *Biston zonaria* with *B. hirtaria*, found that in the hybrids synapsis occurs to a limited extent. Pairing is also noticeable prior to the first maturation division, and such chromosome pairs undergo reduction in a normal manner. The unpaired chromosomes may either divide in each of the two divisions or else go undivided to either pole in one and divide equationally in the other division. The latter thus behaves, as might be expected, like an unpaired chromosome in the spermatogenesis of typical Hemiptera or in the late type of *Oenothera* (Gates, '14). This, however, does not apply to those chromosomes which seemingly are subject to two equation divisions like those of the *Pygaera* hybrids.

Doncaster, observing that the *hirtaria* chromosomes are about four times as large as those of *zonaria*, but that the latter has 56 compared with 14 in *hirtaria*, suggests that the smaller number arises from the larger by a union of chromosomes. In other words, they are compound or multiple chromosomes. If such multiples be made up of unit chromosomes which have the same constitution (and they must bear some such relation to each other if the same ones always join to form the multiples), then in the 56 units of *zonaria* there must be 14 quartets of similar units. It is thus possible that the *Pygaera* chromosomes are really multiple chromosomes which in one of the divisions are merely separated into their component parts. This would then not be a true equation division, for it involves the separation of joined whole chromosomes, neither would it be a true reduction division, as I have mentioned under *Rhodites*. Nevertheless, some such process must take place in all cases where the diploid number of a reduced egg is restored through a reunion of the equational polar body with the nucleus or by a secondary doubling of chromosomes within a cell. All such chromosome sets must be made up of pairs of homologous units. How such multiple chromosomes manage to persist in the maturation of ordinary non-hybrid *Pygaeras* presents still another difficulty, but

this same difficulty exists also in case of the bee where the dyads likewise seem to persist through the maturation.

A few words might be said of the cytological work that Rosenberg ('17) has done on hybrids of the plant genus *Hieracium*. The maturation divisions are in most cases characterized by more or less irregularity. According to Rosenberg, a few of the unpaired chromosomes may undergo two equational divisions, while others are divided only once. But the very irregularity in the amount of pairing and the behavior of single chromosomes must make it practically impossible to be certain in these cases, except when none of the chromosomes are paired and the diploid number of chromosomes is found in each of the four resulting cells after two maturation divisions. Such a case, however, is not mentioned by Rosenberg.

Very puzzling conditions are met with in case of *Dinophilus*. Shearer ('12) described an entrance of the spermatozoa into the oogonia without a fusion of the nuclei. At one of the later divisions he assumed that the male nucleus did not divide with the female nucleus, so that the two daughter cells in one case have a biparental complement of chromatin, in the other a maternal only, the former becoming a female and the latter a male. Nachtsheim ('14), who recently investigated these conditions, finds that Shearer's interpretations are erroneous to a large extent. Thus, there is no precocious entrance of spermatozoa into the oogonia, and unfertilized females may contain female-producing eggs. Instead of the peculiar phenomena described by Shearer, there is a fusion of oocytes, and the male and female eggs apparently differ only in the larger amount of yolk contained in the latter. Possibly the animal is a protandric hermaphrodite and the number of oocytes or nurse cells which join the ultimate germ cell determine whether the male or female state is evolved. How the chromosomal mechanism is correlated with this is by no means clear, especially since Nachtsheim and Shearer both concur in that maturation phenomena are apparently the same in both kinds of eggs (two polar bodies are given off and the diploid number of 20 is reduced to 10). Possibly here also the chro-

mosomes observed in one sex may have a different value from those observed in the other.

The maturation phenomena in the lower Crustacea have not as yet been fully cleared up, although a good deal of work has been done here. However, it seems to be a general rule that parthenogenetic eggs which give rise to parthenogenetic females form only one polar body with no reduction of chromosomes. An exception to this rule was discovered by Brauer ('94) in a small proportion of the parthenogenetic eggs of *Artemia salina*. There two polar bodies were given off and reduction took place, but the second polar body then rejoined the nucleus and restored the diploid number. In the course of later investigations, Fries ('09) did not confirm Brauer in this respect and is inclined to doubt it, while Petrunkevitch ('02) regards the phenomenon as pathological. It is interesting to note in this connection that Artom ('11) has since found that there are two distinct races of *Artemia salina* in Italy, one of which is always sexual, while the other can develop parthenogenetically. The former has eggs which give off two polar bodies and undergo reduction, while the latter form only one polar body with no accompanying reduction. It may be that contradictory results obtained by previous investigators are due to the fact that they were working on similar different races. If crosses could be made between the two races, some light might be thrown on this problem. The fact remains in any case that the diploid complex is retained in some way or other in parthenogenetic eggs which give rise to another parthenogenetic generation.

Sexual eggs give off two polar bodies and reduction occurs. The question of how parthenogenetic sexual eggs which give rise to females (if there really be such) differ from those which give rise to males is not known. From the fact that reduced eggs must be fertilized in order to develop, and that reduction is known to occur in the spermatogenesis of closely related species, it seems likely that the male is not haploid. The male-producing eggs probably extrude certain chromosomes, as do those of Aphids and Phylloxera, and in the spermatogenesis there is perhaps a

process which again duplicates that found at that stage in those groups, only one functional (female-producing) spermatozoon being evolved.

There is no need to enter into details in the well-known cases of Phylloxera and Aphids (v. Baehr, '09; Morgan, '09). Briefly, it might be said that diploid parthenogenesis occurs and only one polar body is given off. In the female eggs this single division is equational, but in the male eggs certain sex chromosomes are cast out entire, and reduction therefore occurs as far as they are concerned.

E. Krüger ('13) has described a case of semiparthenogenesis in the nematode, *Rhabditis aberrans*, in which the egg gives off a single polar body and the division is equational. The spermatozoon enters the egg and starts development, but it does not conjugate with the egg nucleus and finally degenerates. Practically all worms found are hermaphrodites, and males are extremely rare.

In the trematode, *Diplostiscus temporatus* (Cary, '09), parthenogenetic eggs arising in the sporocyst give off only one polar body and no reduction seems to occur, as might be expected. This, of course, is a case of obligatory parthenogenesis.

Finally, mention may be made of artificial parthenogenesis. The starfish egg can be stimulated to develop before the polar bodies have been given off. Maturation will proceed, but according to Buchner ('15) the second polar body rejoins the nucleus, and segmentation stages show the diploid number of chromosomes. Unfortunately, no larvae have been reared to the stage where sex could be determined. Annelid eggs, which are also capable of being stimulated before polar body formation, have likewise not been reared to the necessary stage.

The sea-urchin egg cannot thus be stimulated unless the polar bodies have already been given off. Development therefore proceeds with the haploid number, and Delage has succeeded in raising two such embryos to a stage where he could see that one was certainly a male and the other probably so. Such numbers are of course too small to admit of any definite conclusion.

The fact that development in the frog can be induced artificially has been known for some time (Guyer, '07; Bataillon, '11; Loeb and Bancroft, '13 and others). Without entering into the question as to the nature of such a stimulus, it might be noted that Loeb ('18) reports that he has raised two females and seven males to maturity from such eggs. One of the males examined showed that the number of chromosomes is very probably diploid and certainly not haploid. On this basis Loeb offers as one of several hypotheses that the male is homozygous for sex, while the female is heterozygous, possibly being haploid. This would not explain the production of both sexes, although chromosomal irregularities accentuated by the abnormal method of development may possibly account for this also. But such a hypothesis disregards Swingle's ('17) counts of chromosomes in *Rana pipiens*, which seem to show that the female has twenty-six chromosomes while the males have twenty-five.³

The maturation phenomena in the egg of *Trialeurodes vaporariorum* as it occurs in America probably belong to the group of which the bee is a typical representative, but unlike the latter, does not present complications due to the formation of multiple chromosomes. Conditions shown by the English form, involving the parthenogenetic production of females, very probably resemble the cases mentioned in this discussion, where the diploid number is retained through a reunion of polar body and nucleus or secondary doubling in soma or germ cells. Regarding the spermatogenesis, the entire elimination of the reduction division is not found in any other animal so far investigated, although very often paralleled in the maturation of eggs.

That the two extreme types of chromosomal behavior during maturation should be found within a single order, and even in such closely related families as the Aphidiidae and Aleyrodidae, lends force to the argument that the two types of maturation are not in reality as different as appearances indicate. In the bee type, the diploid female complex naturally has 2 X, while the

³ Loeb has since then raised more frogs that were parthenogenetically produced, and C. L. Parmenter, Jour. Gen. Physiol., vol. 2, who has investigated their cytology, finds that the chromosome number is clearly diploid.

male has 1 X, irrespective of whether the sex chromatin is carried in a single chromosome or not. As far as this chromatin is concerned, such a case does not differ from that of a typical hemipteron with an unmated sex chromosome. No evidence is available that there is in these cases a distinct and separate X element, but in this connection it might be pointed out that coupling of this chromosome with a certain autosome has been observed in a number of cases (Sinety, in *Leptynia*, '02; McClung, in *Orthoptera*, '05; Boveri, '09, and Frolowa, '12, in *Ascaris*, etc.) Neither is the sex chromosome necessarily a single element, as Payne ('12) has pointed out especially in the reduviids. It is thus very possible that each autosome carries a certain amount of sex chromatin. The fact that in the diploid complex the autosomes are doubled as well as the sex chromosomes, if such a condition prevails, and that therefore the proportional relation remains unaltered, may indicate only that the two kinds of chromatin are independent of each other as far as influence on development is concerned. This does not imply that development could proceed in the absence of either of the two kinds of chromatin. It is probable that the autosomal chromatin is active in the causation of sex as well as in the development of all other characters, but this effect is irrespective of its amount involved. At a certain point, however, a decisive factor appears in the influence of the sex chromatin, and this influence, given the conditions already established by the autosomal chromatin, appears to depend on its quantity.

SUMMARY

1. *Trialeurodes vaporariorum* has two races which differ in their parthenogenetic behavior in that the English race gives rise to females and the American to males.
2. In the American race all eggs give off two polar bodies and undergo reduction.
3. Eggs that are not fertilized develop with the haploid number of chromosomes and produce males. Those which are fertilized regain the diploid complex and give rise to females.

4. In the spermatogenesis the haploid complex is retained probably through the entire elimination of the reduction division, and only an equational division occurs.

5. Fertilization of eggs in mated females is apparently under the control of such females, and dependent on some stimulus to which they may be subjected.

6. Discussion of cell inclusions in the Mycetoma.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Early prophase of first maturation division.
- 2 Tetrads.
- 3 Typical plate of first maturation, with eleven condensed tetrads.
- 4 Polar view of the anaphase of the first division.
- 5 Anaphase of the first division.
- 6 Plate of second polar division, with first polar body at the periphery.

Figures 26 to 29 and 36 drawn with no. 6 eyepiece and 1.5-mm. objective.
All others drawn with no. 8 eyepiece and 1.5-mm. objective.

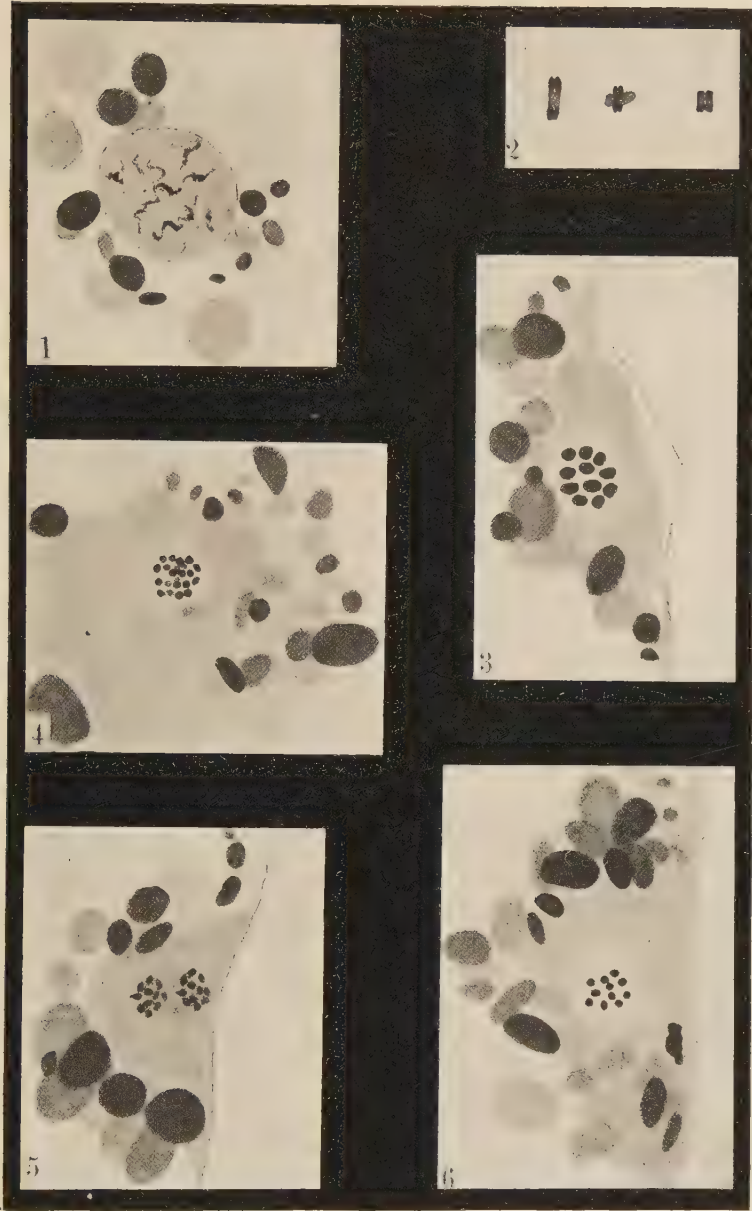


PLATE 2

EXPLANATION OF FIGURES

- 7 Second polar division in metaphase, with first polar body attempting to divide.
- 8 Nucleus of unfertilized egg in semiresting stage. Divided first polar body and the second polar body near periphery. (Two sections.)
- 9 Female pronucleus of a fertilized egg. Second and one of the first polar bodies near the periphery. Sperm head in the interior of the egg. (Two sections.)
- 10 Union of pronuclei (some chromosomes missing).
- 11 Fertilization nucleus with twenty-two chromosomes.

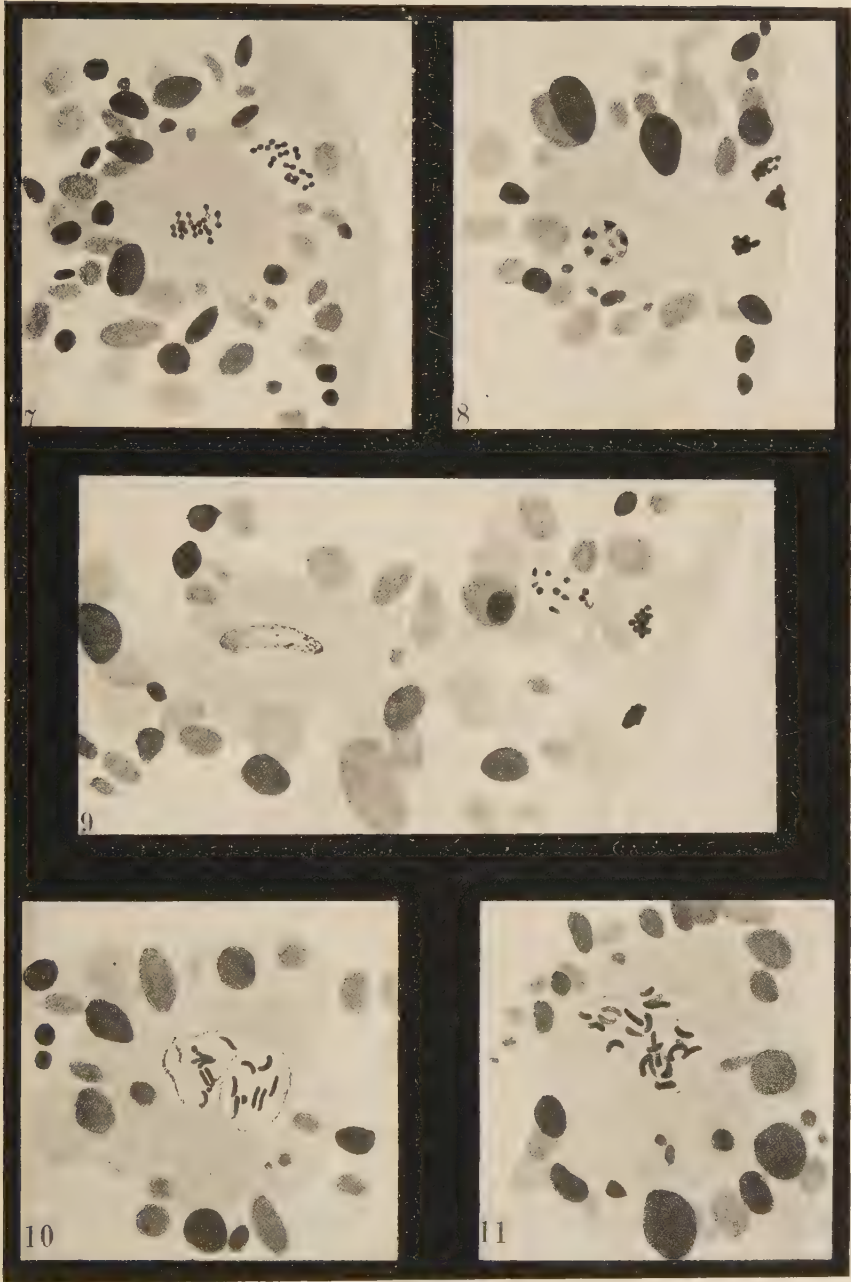


PLATE 3

EXPLANATION OF FIGURES

- 12 Nucleus of unfertilized egg, prior to first somatic division.
- 13 One of the first twelve somatic nuclei showing eleven chromosomes.
- 14 Cell of an early blastula, showing eleven chromosomes.
- 15 Cell in a gastrula stage, with eleven chromosomes.
- 16 Cell in a pupa that has testes developed.
- 17 Cell of an early blastula showing twenty-two chromosomes.
- 18 Cell of an early blastula showing twenty-two chromosomes.
- 19 Cell in a gastrula stage, with twenty-two chromosomes.
- 20 Cell in a pupa that has ovaries developed.
- 21 Division phase of early spermatogonia.
- 22 Division preceding the equational division, probably the last spermatogonial division.
- 23 The equational division.
- 24 Early spermatids.
- 25 Spermatid.

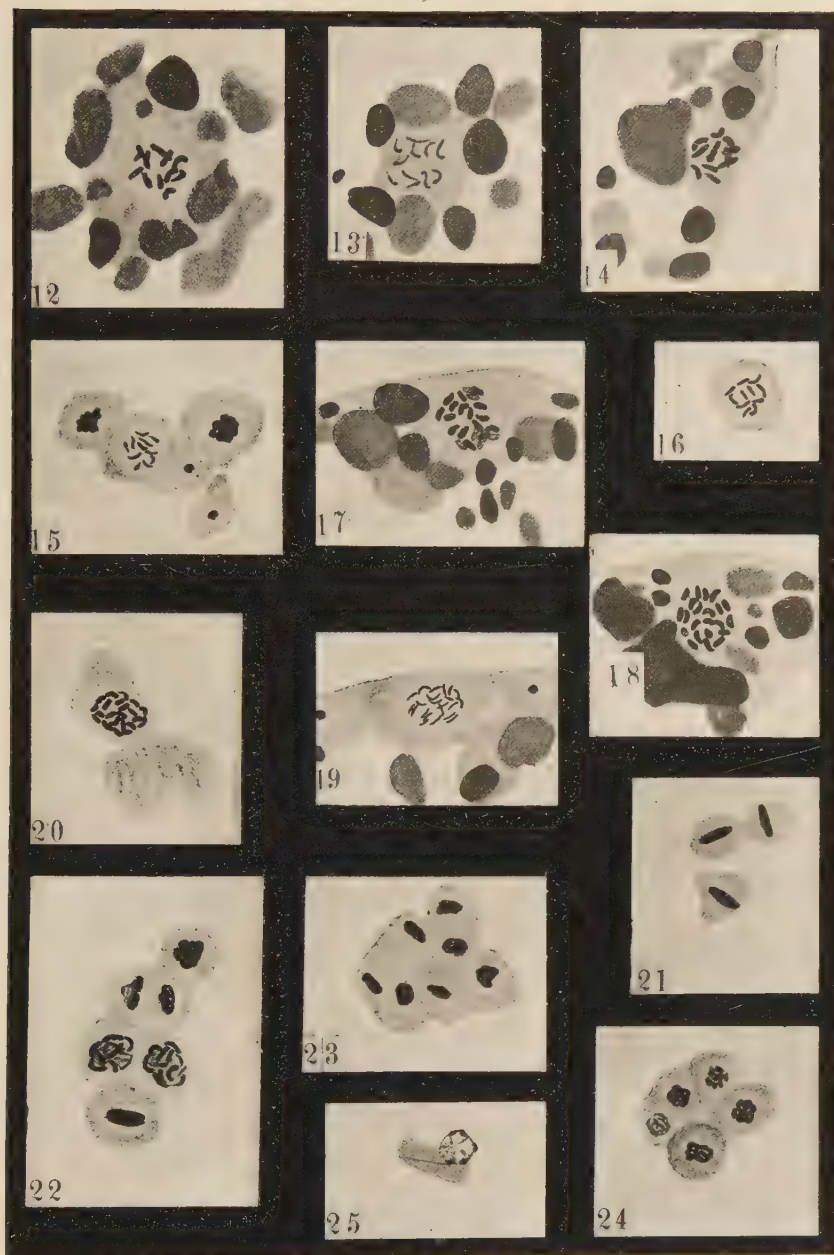
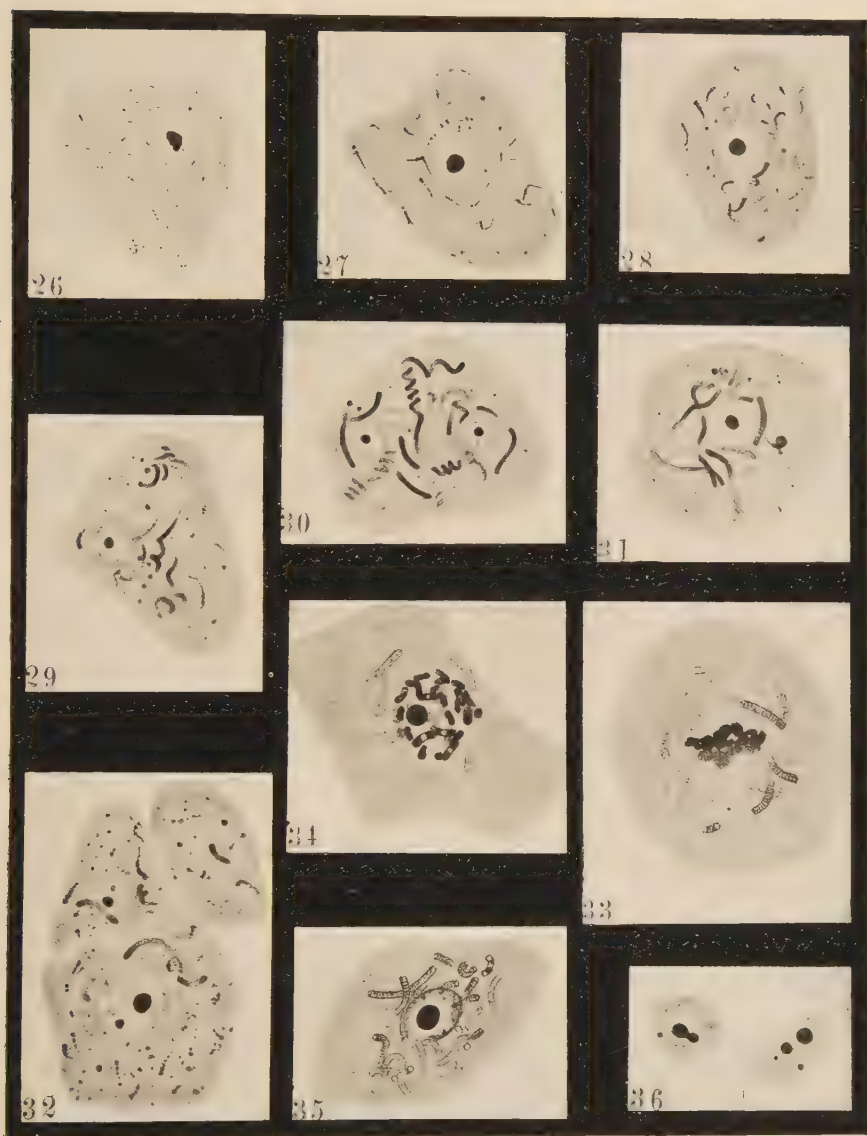


PLATE 4

EXPLANATION OF FIGURES

- 26 Mycetoma cell showing fine granules.
- 27 to 31 Series of stages showing formation of tubules.
- 32 Tubules breaking up.
- 33 Mycetoma cell in division, showing position of tubules at this stage.
- 34 Polar view of mycetoma cell in division.
- 35 Mycetoma cell in resting condition. (Figs. 33, 34, and 35 show effects of fixation with Gilson Carnoy fluid.)
- 36 Examples of budding from the nucleoli.



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